ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Bioactive Food Components and Dietary Supplements

Metabolic Syndrome Is Reduced in C57BL/6J Mice Fed High-Fat Diets Supplemented with Oak Tannins

Ting Luo,[1,2] Tedd Goldfinger,[3] and Neil Shay[2]

[1]State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, China; [2]Food Science and Technology, Oregon State University, Corvallis, OR, USA; and [3]Desert Cardiology of Tucson Heart Center, Tucson, AZ, USA

ABSTRACT

Background: Wine aged in oak barrels will incorporate polyphenols inherent in the staves, suggesting that wine stored in these wooden containers will introduce oak compounds into the human body after consumption.

Objective: The purpose of the present study is to test whether consumption of these oak compounds could favorably influence metabolism in mice fed an obesogenic diet.

Methods: C57BL/6 male mice ($n = 8$) were fed diets for 10 wk as follows: low-fat (LF), high-fat (HF), and HF containing 0.17% of oak tannin (HF+OT). A second 10-wk study was completed; mice were provided LF, HF, and HF diets supplemented with 7.0% of concentrates made from oaked wine (HF+OWC) or unoaked wine (HF+UWC). Physiological parameters were measured during the feeding trial and serum markers and hepatic gene expression measured from samples obtained at necropsy.

Results: Intake of HF+OT significantly reduced body-weight gain (18.4 ± 1.2 g in HF vs. 13.2 ± 1.4 g in HF+OT, $P < 0.05$). Serum resistin concentrations were lower in HF+OT mice compared with HF mice (301 ± 10.1 pg/mL in HF+OT vs. 374 ± 10.9 pg/mL in HF; $P < 0.05$). Hepatic lipid accumulation and expression of glutathione-*S*-transferase-m2 (*Gstm2*) and NAD(P)H:quinone oxidoreductase (*Nqo1*) mRNAs were significantly decreased in HF+OT compared with HF mice ($P < 0.05$). When compared with HF-fed mice, intake of both OWC and UWC decreased body-weight gain ($P < 0.05$), with no significant impact on food consumption. Fasting glucose concentrations, serum insulin, and hepatic lipid accumulation were reduced in HF+OWC-fed mice compared with HF+UWC-fed mice ($P < 0.05$). Furthermore, hepatic glutathione-*S*-transferase-a1 (*Gsta1*) mRNA levels were significantly reduced in OWC-supplemented (0.25 ± 0.08) compared with UWC-supplemented (1.71 ± 0.24) mice ($P < 0.05$).

Conclusions: In this mouse model of metabolic disease, intake of OTs and a concentrate made from an oaked wine had a potent impact on alleviating HF-induced metabolic syndrome. Thus, intake of OTs, provided passively in oaked wine or as a dietary supplement, may act as an agent to attenuate the markers of metabolic syndrome. *Curr Dev Nutr* 2020;00:nzaa033.

Keywords: oak tannins, oaked wine, polyphenols, metabolic syndrome, obesity, diabetes, mouse, high fat

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Address correspondence to NS (e-mail: neil.shay@oregonstate.edu).

Abbreviations used: AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; *Cyp*, cytochrome P450; *Gsta1*, glutathione-*S*-transferase-a1; *Gstm2*, glutathione-*S*-transferase-m2; HF, high-fat; *Hmox1*, heme oxygenase-1; LF, low-fat; MCP-1, monocyte chemoattractant protein-1; *Nqo1*, NAD(P)H:quinone oxidoreductase; Nrf2, nuclear factor erythroid 2–related factor 2; OT, oak tannin; OWC, oaked wine concentrate; PPAR, peroxisome proliferator activated receptor; PXR, pregnane X receptor; *Scd1*, sterol CoA desaturase-1; UWC, unoaked wine concentrate.

Introduction

Metabolic syndrome is defined as a cluster of 5 metabolic abnormalities, including obesity, elevated triglycerides, low HDL cholesterol, hypertension, and hyperglycemia. It is one of the most significant health problems worldwide (1). The presence of metabolic syndrome criteria is associated with cardiovascular disease and affects >20% of the population of the United States (2, 3).

Management of metabolic syndrome involves a combination of lifestyle changes, including diet- and/or physical activity–based interventions and pharmacological interventions. Various natural compounds derived from food or plant extracts may produce a beneficial effect on the management of metabolic syndrome (3). Previously, we have demonstrated that intake of bioactive compounds including soy isoflavones (4), ellagic acid and raspberry ketone (5), quercetin (6), and resveratrol (7) have been correlated with improvements in metabolism when studied in mouse models of obesity and metabolic disease.

Tannins are oligomeric and polymeric forms of various polyphenols and, in addition to known fruit and vegetable sources, are also derived from oak and other wood products. Wood tannins may contribute to

TABLE 1 Composition of C57BL/6J male mice diets, including LF, HF, HF+OT, HF+OWC, and HF+UWC[1]

	LF	HF	HF+OT	HF+OWC	HF+UWC
Ingredients, g					
Casein	200	200	200	200	200
l-Cysteine	3	3	3	3	3
Corn starch	506.2	72.8	72.8	72.8	72.8
Maltodextrin 10	125	100	100	100	100
Sucrose	68.8	172.8	172.8	172.8	172.8
Cellulose, BW200	50	50	50	50	50
Soybean oil	25	25	25	25	25
Lard	20	177.5	177.5	177.5	177.5
Mineral mix S10026	10	10	10	10	10
Dicalcium phosphate	13	13	13	13	13
Calcium carbonate	5.5	5.5	5.5	5.5	5.5
Potassium citrate, $^{1}H_2O$	16.5	16.5	16.5	16.5	16.5
Vitamin mix V10001	10	10	10	10	10
Choline bitartrate	2	2	2	2	2
Cholesterol	0	8.5	8.5	8.5	8.5
OTs	0	0	1.5	0	0
OWC	0	0	0	65.3	0
UWC	0	0	0	0	65.3
Percent of energy, kcal%					
Protein	20	18	18	18	18
Carbohydrate	70	36	36	36	36
Fat	10	46	46	46	46
kcal/g	3.8	4.59	4.59	4.59	4.59

[1]Based on the phenolic content of the OT powder and the 2 concentrates, the total phenolic contents of the test diets are as follows: OT, 0.089%; OWC, 0.135%; UWC, 0.068% (all wt/wt). HF, high-fat; LF, low-fat; OT, oak tannin; OWC, oaked wine concentrate; UWC, unoaked wine concentrate.

the hypothesized beneficial effects of wine consumption on the development of metabolic syndrome and cardiovascular disease. In 1999, a prospective cohort study in 36,250 healthy men from France had already demonstrated that a regular, moderate consumption of red wine (22–32 g alcohol) was associated with a reduced risk of cardiovascular death (8). At that time, the components accounting for the beneficial effect were unclear. Another report from 2006 found that people in areas of southwestern France and Sardinia enjoyed greater longevity, which might be because of a different vinification practice in these areas, resulting in a higher procyanidin content of the local wine (9).

Tannins are the major component in wine phenolics and can make up as much as 50% of the total phenolics. Oak-derived polyphenols often are oligomers of gallic and ellagic acid and include roburin, vescalagin, castalagin, grandinin, and vescalin (10). They appear in wine mainly from aging in oak barrels or in contact with grape skins and seeds during fermentation. Tannin is defined as water-soluble phenolic compounds having a molecular weight between 500 and 3000 Da for gallic acid esters, while proanthocyanin tannins will range up to 20,000 Da (11). Tannins are composed of flavonoid and nonflavonoid constituents, including anthocyanins, dihydroflavonols, flavanol monomers (catechins) and polymers (proanthocyanidins), flavonols, phenolic acids, and stilbenoids. The amount and composition of the tannins in wine impact the color and mouthfeel of the wine.

We hypothesize that the consumption of oak tannins (OTs) would produce beneficial effects on the consequences of consumption of an obesogenic high-fat (HF) diet in mice. The consumption of purified OTs (study 1) or an oaked wine concentrate (OWC; study 2) may alleviate HF-diet–induced body-weight gain and hepatic lipid accumulation and change gene expression related to lipid and glucose metabolism in this mouse model. This study aims to provide new information on health benefits of polyphenols derived from oak, which may be consumed either with wine or as a dietary supplement product.

Methods

C57BL/6J mice and diets

In study 1, 6-wk-old male C57BL/6J mice (Jackson Laboratories) were acclimated to our facility and fed a semipurified low-fat (LF) diet for 2 wk. Twenty-four mice were then randomly divided into 3 groups ($n = 8$). Two control groups were fed either the LF diet, containing 10% fat and 70% carbohydrate by energy, or an HF control diet containing 45% fat with 1% cholesterol. A third group was fed the HF diet with 0.17% (wt/wt) of OT powder (HF+OT) added. OT powder was obtained from Scott Laboratories (Tannin Riche, no. 015962). This product is a 100% oak product produced from aqueous processing of toasted French oak (*Quercus robur*). Mice were fed these 3 diets ad libitum for 10 wk. For mouse study 2, 32 mice were divided into 4 groups, including LF, HF, HF+OWC, or HF + unoaked wine concentrate (UWC) (**Table 1**). The OWC and UWC were produced from oaked (*Q. robur*) and unoaked Barbera wine, respectively (Brezza Winery, Barolo, Italy). Wines were freeze-dried overnight and the resulting alcohol-free solid was rehydrated into a minimum volume of water before mixing into experimental diets. Diets for both trials were produced by Research Diets, Inc.

In both studies, mice were kept 4 per cage in a room maintained at a constant temperature (24°C), with a 12-h light-dark cycle, and given free access to diet and distilled water. During the 10-wk feeding trial,

TABLE 2 Total phenolics in OT powder, OWC, and UWC[1]

Sample ID	Concentration, g/100 g
OT	52.2
OWC	1.92
UWC	0.98

[1] OT, oak tannin; OWC, oaked wine concentrate; UWC, unoaked wine concentrate.

body weight and food intake were recorded once per week, with spillage accounted for. The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC 4455).

Quantification of phytochemicals by HPLC

Total phenolic compounds in OTs, OWC, and UWC were measured by the Folin-Ciocalteu spectrophotometric method, as described previously Snyder et al. (6). Phenolic compounds were analyzed by HPLC with the use of the method that has been proposed as an AOAC method for phenolic compound analysis. The content of individual compounds in extracts was determined qualitatively by comparison of HPLC chromatograms of extracts and mixtures of phytochemical standards.

Fasting blood glucose and intraperitoneal glucose-tolerance test

In week 9 of the feeding trial, tail-vein blood glucose concentrations were measured. Diet was withheld for 6 h prior to testing and the test was performed midday in the middle of the light cycle. In study 1, mice were administered glucose (0.1 mg/g body weight) by intraperitoneal injection. Blood glucose concentrations were measured from a droplet of blood obtained from the tail vein using a handheld glucometer (ReliOn; Abbott Laboratories). In mouse study 1, glucose concentrations were measured at 0, 15, 30, 60, 90, and 120 min after glucose injection. The index of glucose tolerance was indicated as the AUC using the trapezoidal rule to determine AUC (12, 13). In study 2, the intraperitoneal glucose-tolerance test was not performed; only a single baseline glucose measurement was taken.

Plasma biomarker quantitation

Blood samples were collected via cardiac puncture, incubated on ice for 30–60 min, and centrifuged at 1000 × *g* for 15 min at 4°C, after which serum was collected. Plasma biomarkers were measured in 96-well plates using Milliplex® MAP kits (Millipore). Parameters in the plasma including insulin, resistin, and monocyte chemoattractant protein-1 (MCP-1) were measured. Plates were read on a Luminex 200 instrument (Luminex) and analyzed using xPONENT software (Luminex).

Histological analysis of liver tissue

After 10 wk of feeding experimental diets, mice were killed by cardiac puncture exsanguination after anesthetization with isoflurane, and liver tissue was extracted. Liver tissue was fixed in buffered formalin and paraffin embedded, and three to four 5-μm-thick sections were transferred to numbered slides. Slides were then stained with Masson's trichrome stains. Images were acquired using a Nikon Eclipse E400 microscope (Nikon Co.) equipped with an extended digital camera (Q Imaging). Four different fields per mouse liver were analyzed for hepatic lipid accumulation. Lipid droplet percentage (the ratio of white color area to the total area) was obtained with Adobe Photoshop 7.0, generally following guidelines by Dahab et al. (14).

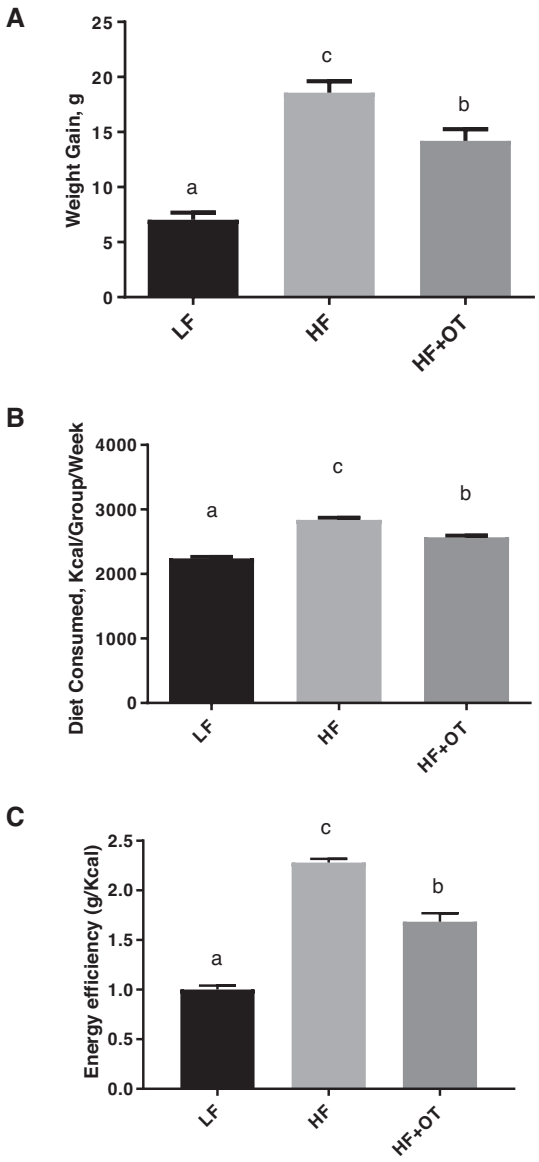

FIGURE 1 Body-weight gain at week 10, energy intake per group per week, and energy efficiency in C57BL/6J male mice fed an LF, HF, and HF plus 0.17% (wt/wt) OT powder (HF+OT) diet. (A) Body-weight gain of mice fed an LF, HF, HF+OT diet at week 10. (B) Diet consumption of mice per group per week in LF, HF, and HF+OT groups. (C) Energy efficiency [energy efficiency = body weight gained (g)/total energy intake (kcal) × 100%] of mice in the LF, or HF, or HF+OT groups. Bars are means ± SEMs ($n = 8$). Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin.

Hepatic gene expression determination

Liver samples were dissected, snap frozen, and stored at −80°C until use. RNA was isolated from using TRIzol reagent (Invitrogen Life Technologies). RNA was reverse-transcribed with the PrimerScript™

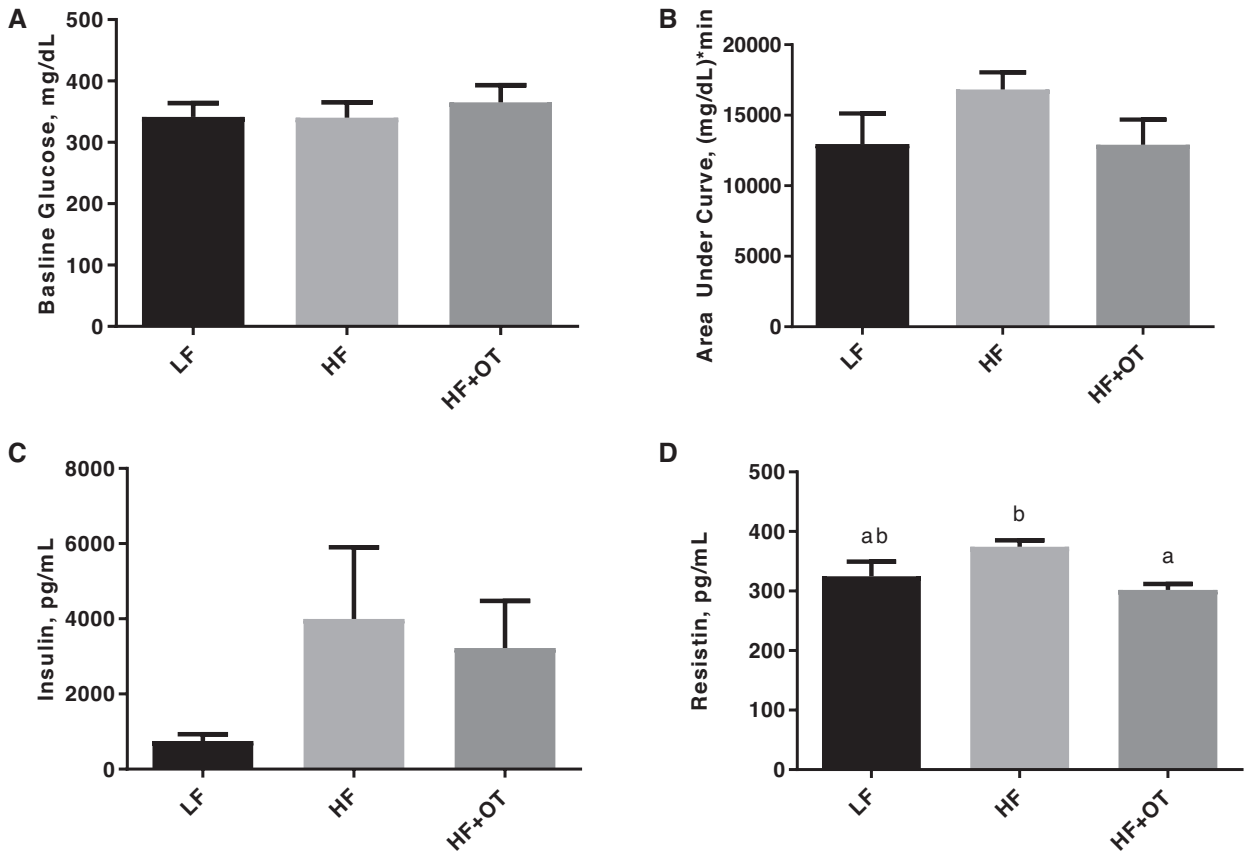

FIGURE 2 Parameters of glucose homeostasis in C57BL/6J male mice fed an LF, HF, and HF plus 0.17% (wt/wt) OT powder (HF+OT). (A) Baseline glucose concentration. Blood glucose concentrations were measured from the tail vein using a handheld glucometer. (B) AUC from time 0 to time 120 min after glucose injection. Plasma glucose concentrations were determined 0, 15, 30, 60, 90, and 120 min after glucose injection (0.1 mg/g body weight glucose). (C) Serum insulin concentration. (D) Serum resistin concentration. Bars are means ± SEMs ($n = 8$). Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin.

RT-PCR kit (Takara) according to the manufacturer's protocol. The relative mRNA levels for specific genes were determined by real-time PCR using SYBR Premix Ex Taq™ (Applied Biosystems). PCR products were quantified using the ABI 7900HT real-time PCR system (Applied Biosystems). Gene expression levels were normalized to the housekeeping gene *Gapdh*. Real-time PCR was performed as follows: 95°C for 10 min, then 40 cycles of 95°C for 5 s and 60°C for 20 s.

Statistical analysis

Data are presented as means ± SEMs. ANOVA was used to compare sets of data. Tukey's procedure was used for the post hoc testing. Significance was established at a level of $P < 0.05$, and a P value between 0.05 and 0.10 is considered as a trend toward significance. All statistical analyses were carried out using GraphPad Prism 6 (Graph Pad).

Results

Total phenolics in OT, OWC, and UWC

As shown in **Table 2**, per gram, OT has the highest total phenolics, followed by OWC, and then UWC.

Mouse study 1

Effects of OT on body weight in HF-fed mice.

Compared with LF-fed mice, HF-fed mice had significantly increased body-weight gain (7.08 ± 0.9 g in LF vs. 18.4 ± 1.2 g in HF; $P < 0.05$). However, the body-weight gain in HF+OT-fed mice (13.2 ± 1.4 g) was decreased ($P < 0.05$) compared with HF-diet–fed mice (**Figure 1**A). Energy intake in HF-diet fed mice was higher than that in LF- and OT-fed mice ($P < 0.05$). Energy intake in HF+OT-fed mice was intermediate between LF- and HF-diet-fed mice and was significantly different from both LF and HF ($P < 0.05$; Figure 1B). As shown in Figure 1C, compared with the HF group, energy efficiency [energy efficiency = body weight gained (g)/total energy intake (kcal) × 100%] was also significantly reduced in HF+OT-fed mice ($P < 0.05$).

Effects of OT on glucose homeostasis.

No difference in baseline blood glucose concentrations was observed in LF-, HF-, and HF+OT-fed mice ($P = 0.76$; **Figure 2**A). We examined the effects of dietary OT on glucose tolerance: in the HF-diet group, AUC was higher than that in LF-diet-fed mice. There was a

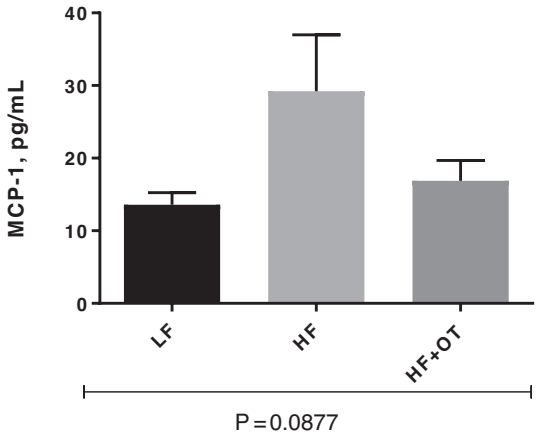

FIGURE 3 Serum concentrations of MCP-1 in C57BL/6J male mice fed an LF, HF, and HF plus 0.17% (wt/wt) OT powder (HF+OT) at week 10. The effect of OT on serum MCP-1 concentration was measured by ELISA. Bars are means ± SEMs. Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; MCP-1, monocyte chemoattractant protein-1; OT, oak tannin.

trend that OT supplementation ameliorated overall glucose tolerance ($P = 0.16$; Figure 2B).

No significant difference in serum insulin concentration was found in mice in the LF, HF, and HF+OT groups (Figure 2C). However, in HF+OT- versus HF-fed mice, serum resistin concentrations were significantly decreased (374 ± 10.9 pg/mL in HF vs. 301 ± 10.1 pg/mL in HF+OT; $P < 0.05$). Resistin concentrations for the HF+OT mice were statistically equivalent to the LF-fed group (Figure 2D).

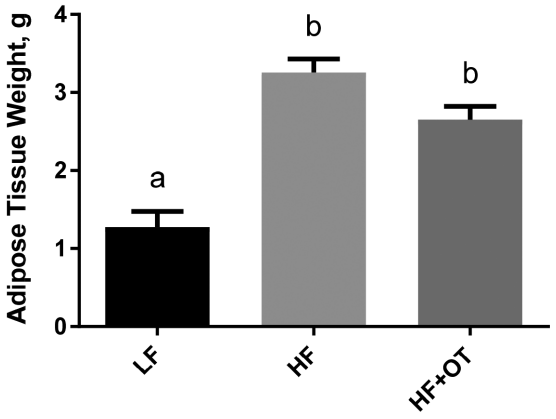

FIGURE 5 Adipose tissue weight of C57BL/6J mice fed an LF, HF, and HF plus 0.17% (wt/wt) OT powder (HF+OT) at week 10. Bars are means ± SEMs. Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin.

Effect of OT intake on MCP-1.

There was a trend toward significance for serum MCP-1 concentrations in HF+OT- compared with HF-fed mice ($P = 0.09$; **Figure 3**).

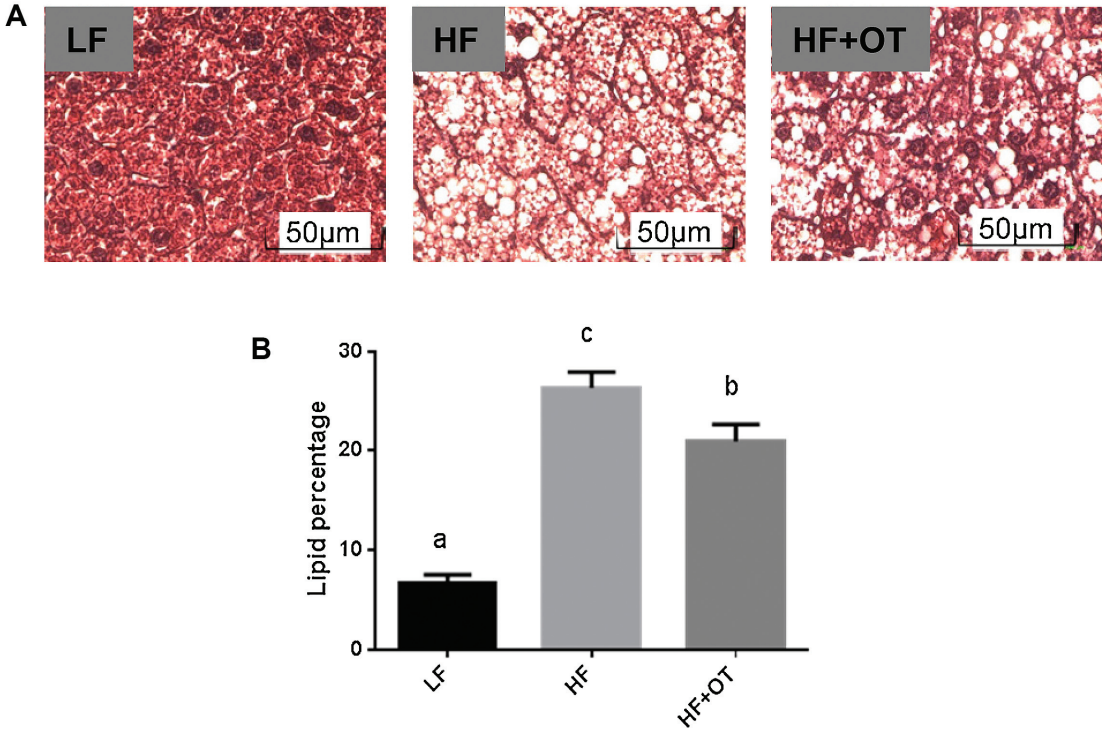

FIGURE 4 Hepatic lipid accumulation in C57BL/6J male mice fed an LF, HF, and HF plus 0.17% (wt/wt) OT powder (HF+OT) at week 10. (A) Representative photomicrographs of stained liver sections from mice fed LF, HF, and HF+OT diets. (B) Quantified results of lipid accumulation in LF, HF, and HF+OT groups. Bars are means ± SEMs ($n = 8$). Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin.

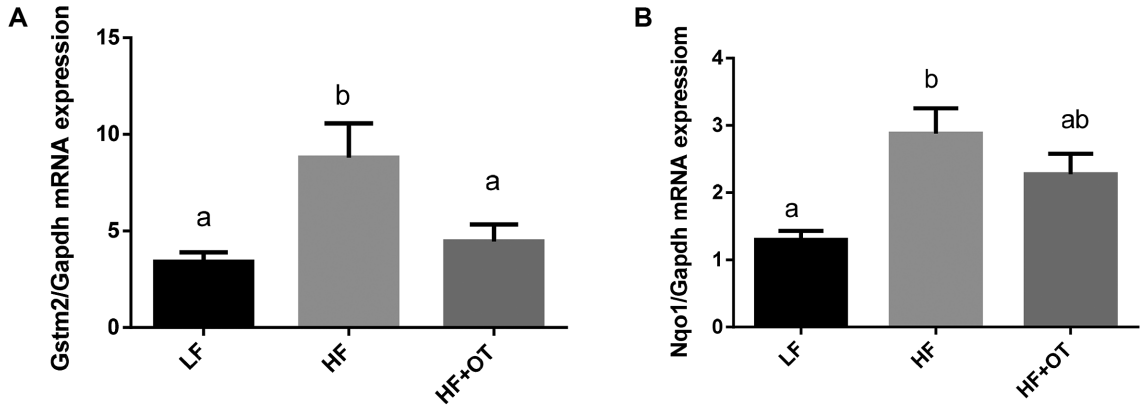

FIGURE 6 Hepatic gene expression of C57BL/6J mice fed an LF, HF, and HF plus 0.17% (wt/wt) OT powder (HF+OT) at week 10. Gene expression of *Gstm2* (A) and *Nqo1* (B) was determined by real-time PCR. Expression is relative to the *Gapdh* housekeeping gene. Bars are means ± SEMs ($n = 8$). Bars sharing the same letter are not significantly different from each other, $P < 0.05$. *Gstm2*, glutathione-*S*-transferase-m2; HF, high-fat; LF, low-fat; *Nqo1*, NAD(P)H:quinone oxidoreductase; OT, oak tannin.

Effects of OT intake on lipid accumulation.
HF-fed mice had the greatest liver lipid content compared with the other 2 diet groups ($P < 0.05$). The HF+OT-fed mice showed a reduction in hepatic lipid accumulation compared with HF-fed mice. LF-fed mice had the lowest lipid content compared with the other 2 groups (all $P < 0.05$; **Figure 4**).

Effects of OT intake on adipose tissue weight in HF-fed mice.
Inguinal fat-pad weights were measured at necropsy. As shown in **Figure 5**, OT intake had no significant effect on adipose tissue weight versus the HF diet.

Effects of OT on hepatic gene expression.
Relative glutathione-*S*-transferase-m2 (*Gstm2*) mRNA levels in both LF- and HF+OT-fed mice were significantly decreased compared with HF-fed mice ($P < 0.05$; **Figure 6**A). The relative *Nqo1* mRNA level in HF+OT-fed mice was reduced compared with HF-fed mice, such that they were statistically equivalent to the LF-fed mice (Figure 6B).

Mouse study 2

Effects of OWC or UWC on body weight in HF-fed mice.
Intake of the HF diet robustly increased mouse body weight gain after 10 wk (5.07 ± 0.6 g in LF vs. 16.8 ± 1.7 g in HF; $P < 0.05$). Compared with HF-fed mice, consumption of either OWC or UWC diets significantly decreased mouse body weight versus the HF diet ($P < 0.05$; **Figure 7**).

No significantly different diet consumption was observed in HF, HF+OWC, and HF+UWC groups, while LF-fed mice consumed less energy than all HF-fed mice (Figure 7B). Compared with the HF group, energy efficiency was significantly decreased in the HF+OWC- and HF+UWC-fed groups ($P < 0.05$; Figure 7C).

Effects of OWC or UWC on glucose homeostasis in HF-fed mice.
The supplementation of OWC in the HF diet decreased mouse baseline glucose concentrations compared with both HF- and HF+UWC-fed groups ($P < 0.05$). There was no significant difference in baseline glucose concentrations in HF-, and HF+UWC-fed mice (**Figure 8**A). In a similar fashion, serum insulin concentration was decreased in HF+OWC-fed mice compared with HF- and HF+UWC-fed mice ($P < 0.05$). The HF- and HF+UWC-fed mice had statistically equivalent insulin concentrations while HF+UWC-fed mice had insulin concentrations statistically equivalent to both LF- and HF+OWC-fed mice.

Effects of OWC or UWC on lipid accumulation in HF-fed mice.
Liver lipid concentrations in LF-fed mice were the lowest. HF+OWC-fed mice had concentrations that were statistically equivalent to both LF- and HF-fed mice. The groups with the highest amounts of hepatic fat were the HF and HF+UWC groups. Relative hepatic lipid percentages were 9.2% ± 1.3% (LF), 29.8% ± 3.5% (HF), 15.0% ± 3.3% (HF+UWC), and 32.1% ± 4.2% (HF+UWC) (**Figure 9**).

Effects of OWC or UWC on adipose tissue weight in HF-fed mice.
Compared with LF-fed mice, adipose weight was significantly increased in HF-diet-fed mice ($P < 0.05$). In mice fed the HF diet with OWC or UWC, there were no significant differences compared with HF-fed mice (**Figure 10**).

Effects of OWC or UWC on hepatic gene expression.
Relative gene expression of glutathione-*S*-transferase-a1 (*Gsta1*) was significantly increased in mice fed the HF diet compared with mice in the LF group ($P < 0.05$; **Figure 11**). In HF+OWC-fed mice, there was a reduction in *Gsta1* gene expression that was significantly lower than in HF-fed mice ($P < 0.05$), and equivalent to that in LF-fed mice.

Discussion

With intake of HF+OT (study 1) and intakes of HF+OWC and HF+UWC (study 2), significant decreases in both body-weight gain and energy intake were observed. The cause for the reduced intake is not fully understood, but could be caused, in part, by something as simple as a reduced palatability of the diet because of the taste of added tan-

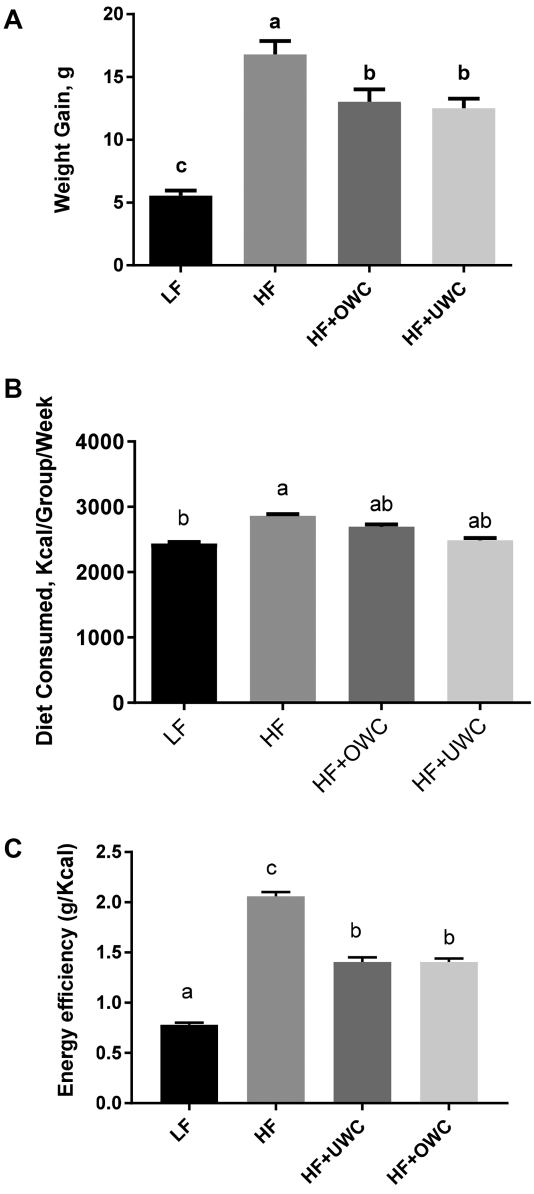

FIGURE 7 Body-weight gain at week 10, energy intake per group per week, and energy efficiency in C57BL/6J male mice fed LF, HF, HF+OWC, or HF+UWC diets. (A) Body-weight gain of mice fed an LF, HF, HF+OWC, and HF+UWC diet at week 10. (B) Diet consumption of mice per group per week in LF, HF, HF+OWC, and HF+UWC groups. (C) Energy efficiency [energy efficiency = body weight gained (g)/total energy intake (kcal) × 100%] of mice in LF, or HF, HF+OWC, or HF+UWC groups. Bars are means ± SEMs ($n = 8$). Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin; OWC, oaked wine concentrate; UWC, unoaked wine concentrate.

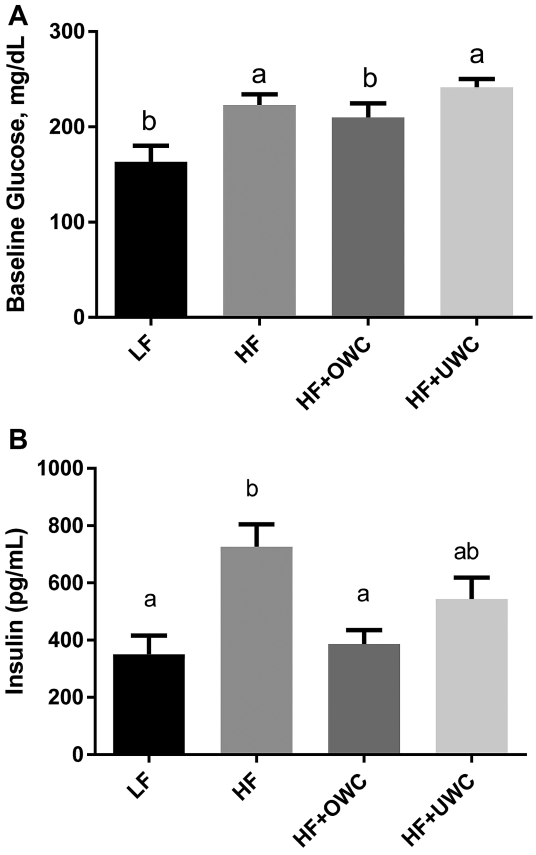

FIGURE 8 Parameters of glucose homeostasis in C57BL/6J male mice fed an LF, HF, HF+OWC, or HF+UWC diet. (A) Baseline serum glucose concentrations. Blood glucose concentrations were measured from the tail vein using a handheld glucometer, after 6 h of feed deprivation. (B) Plasma insulin concentration. Bars are means ± SEMs ($n = 8$). Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin; OWC, oaked wine concentrate; UWC, unoaked wine concentrate.

nins to the HF diet. However, inspection of the energy efficiencies in these 2 studies demonstrate that energy efficiency is decreased significantly in both studies (Figures 1 and 7). This supports the hypothesis that body metabolism in the HF-fed groups is changed in groups fed oak and wine tannins. In particular, study 2 shows that the energy intake is only very slightly different, whereas more profound differences in weight gain appear to be driving the differences in energy efficiency. Some of these questions could be answered in future studies involving taste preference and metabolic cage testing to determine metabolic rate and respiratory quotient. Inguinal fat-pad weights generally followed body-weight gain patterns (Figures 5 and 10). In our own laboratory, we found a tight correlation of overall adiposity in rodents and inguinal fat weight (data not shown). It is also possible that exposure to oak phytochemicals changes the microbiome populations, and that these changes are part of the observed differences in energy efficiency. Future work should include microbiome quantification.

Glucose metabolism was impacted with the consumption of OT, OWC, and UWC. In study 1, no statistically significant findings were reported for baseline glucose, glucose AUC, or insulin. We measured insulin values to be ~5-fold greater in HF- versus LF-fed mice, and 4-fold greater in HF+OT- versus LF-fed mice ($P = 0.29$). This relatively high P value was a consequence of great variation in serum insulin concentrations in the HF- and HF+OT-fed mice. However, serum resistin concentrations were reduced in HF+OT- versus HF-fed mice

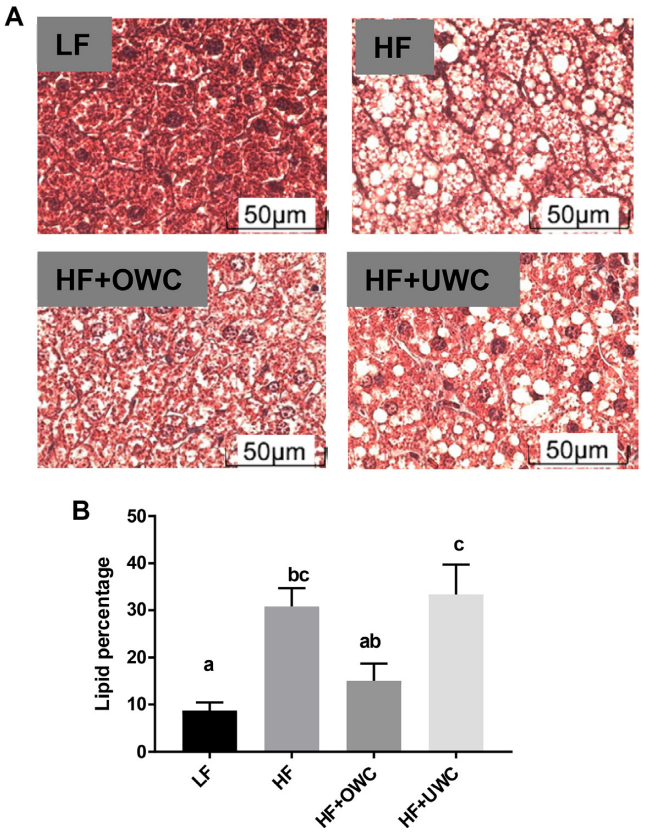

FIGURE 9 Hepatic lipid accumulation in C57BL/6J male mice fed an LF, HF, HF+OWC, or HF+UWC diet at week 10. (A) Representative photomicrographs of liver sections stained with hematoxylin & eosin for the indicated diet groups: LF, HF, HF+OWC, and HF+UWC. (B) Quantified results of lipid accumulation in LF-, HF-, HF+OWC-, and HF+UWC-fed mice. Bars are means ± SEMs ($n = 8$). Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin; OWC, oaked wine concentrate; UWC, unoaked wine concentrate.

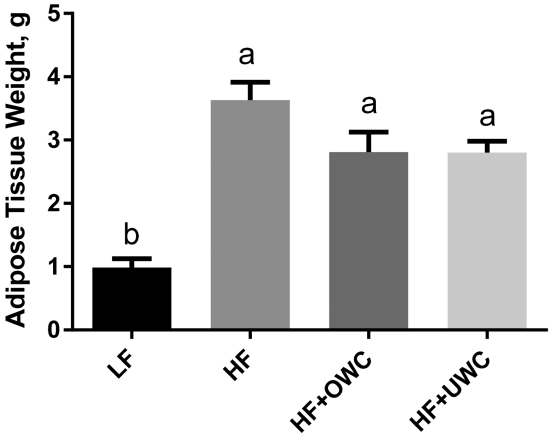

FIGURE 10 Adipose tissue weight of C57BL/6J mice fed an LF, HF, HF+OWC, or HF+UWC diet at week 10. Inguinal fat-pad weight was measured at necropsy. Values are means ± SEMs. Bars sharing the same letter are not significantly different from each other, $P < 0.05$. HF, high-fat; LF, low-fat; OT, oak tannin; OWC, oaked wine concentrate; UWC, unoaked wine concentrate.

($P < 0.05$; Figure 2). Resistin is linked to obesity and predictive of atherosclerosis in humans. It has been reported that circulating concentrations of resistin are elevated in obese and insulin-resistant rodents and humans (15). In study 2, mice fed HF+OWC had a reduced baseline glucose concentration that was statistically equivalent to that in LF-fed mice. The HF+UWC-fed mice did not share this reduction, suggesting a component in OWC, not present in UWC, is improving glucose sensitivity. Serum insulin concentrations showed similar results, with a reduction in insulin in HF+OWC-fed mice, such that their insulin concentration was essentially the same as that in LF-fed mice. In study 2, HF+UWC-fed mice did show a partial reduction in insulin, such that the concentration became statistically indistinguishable from that in the LF group. One potential explanation for the observed changes for insulin would be if OTs in the OT and OWC diets, and perhaps to a lesser degree, grape compounds in the UWC diet had an agonistic activity on peroxisome proliferator activated receptor (PPAR) γ (PPARγ) signaling (16). Activation of PPARγ signaling is known to improve insulin sensitivity (17). Resistin concentrations were not measured in study 2 due to a lack of sufficient mouse serum.

A marker of inflammation, MCP-1, was marginally impacted by diet in study 1 ($P = 0.09$; Figure 3) and was not tested in study 2. However, hepatic fat content was measured in both studies and was reduced by the presence of OTs in both studies (HF+OT vs. HF in study 1, and HF+OWC vs. HF in study 2). Intake of HF+UWC did not reduce hepatic fat concentrations compared with HF-fed mice. One possible explanation for this finding would be that OTs, present in both OT and OWC diets, have an impact on hepatic β-oxidation that UWC does not share. One possible activity would be the action on the hepatic PPARα receptor. Activation of PPARα induces uptake of fatty acids into the mitochondria, and their catabolism into acetate and ATP (18, 19).

To try to better understand the impact of OT consumption, a limited number of mRNAs were evaluated for expression using RT-PCR. In study 1, *Gstm2* and *Nqo1* levels were measured. Both mRNAs can be positively regulated by nuclear factor erythroid 2–related factor 2 (Nrf2). Further, *Nqo1* can be transcriptionally upregulated by aryl hydrocarbon receptor (AhR) as well, while *Gstm2* is also regulated by the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) (20). Data displayed in Figure 6 are consistent with upregulation of these 2 mRNAs with consumption of the HF diet, with a partial or complete reduction of that increase with the consumption of the HF+OT diet. One potential explanation for this pattern is an upregulation of *Nrf2* on transcription of these 2 target genes, related to a pro-oxidant state associated with consumption of the HF diet. This pro-oxidant state may then be partially (*Nqo1*) or completely (*Gstm2*) restored with the addition of OT to the HF diet. This explanation may be preferred to alternate explanations involving AhR, CAR, or PXR, as it would seem the phytochemicals in OT, acting as xenobiotics, would up- and not downregulate relative gene levels in the HF+OT-fed mice.

In study 2, *Gsta1* mRNA levels were measured. In male mice, *Gsta1* levels are regulated strongly by Nrf2 and less so by PXR and CAR (20). Again, our data are consistent with upregulation of Nrf2 action with

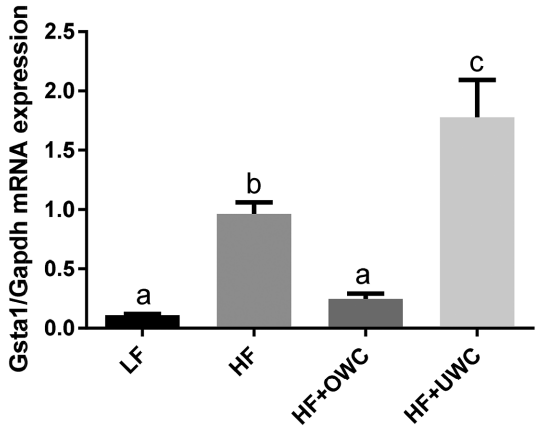

FIGURE 11 Hepatic gene expression of C57BL/6J mice fed an LF, HF, HF+OWC, or HF+UWC diet at week 10. Gene expression of *Gsta1* was determined by real-time PCR. Expression is relative to the *Gapdh* housekeeping gene. Bars sharing the same letter are not significantly different from each other, $P < 0.05$. *Gsta1*, glutathione-*S*-transferase-a1; HF, high-fat; LF, low-fat; OT, oak tannin; OWC, oaked wine concentrate; UWC, unoaked wine concentrate.

consumption of the pro-oxidant HF diet, and restoration to LF-fed levels with OWC but not UWC (Figure 11).

Other mRNAs measured in study 1 that were upregulated with HF diet consumption and then restored to LF levels in HF+OT mice were sterol CoA desaturase-1 (*Scd1*) and cytochrome P450 (*Cyp*) 2b10 (*Cyp2b10*) (data not shown). In male mice, *Cyp2b10* mRNA levels appear to be profoundly regulated by CAR and to a lesser degree Nrf2. Transcription of the *Scd1* gene is more complex but is quite sensitive to diet. In study 2, other mRNAs were measured, including *Cyp1a2, Cyp2b10,* and heme oxygenase-1 (*Hmox1*). For all 3 of these mRNAs, relative levels were higher ($P < 0.05$) in HF+OWC- versus HF-fed mice, while HF+UWC-fed mice had mRNA levels statistically equivalent to those in HF-fed mice (data not shown). *Cyp1a2* is profoundly regulated by AhR and the pattern of expression in LF, HF, HF+OWC, and HF+UWC is consistent with AhR activation by OT. *Hmox1* transcription may be more complex, but may be partly regulated, again by Nrf2. In this case, however, it appears that Nrf2 is being activated by OT. The reason for the differences between the pattern of expression for *Hmox1* and the other Nrf2-regulated mRNAs (e.g., the *Gst*'s) is not clear and likely involves other regulatory factors.

To better understand transcriptional changes caused by consumption of OT, OWC, and UWC, we suggest that gene profiling, via RNASeq, for example, would provide a much clearer overall view of the impact of these phytochemicals. Also, the use of knockout mouse strains or cell lines for some of these factors (e.g., Nrf2, AhR, and CAR) would be informative and interesting.

Other major issues that should be addressed include the amount of dietary dose for OT, OWC, and UWC. The design of this study was not to simulate the effect of OTs at an amount provided by drinking an oaked wine, but rather the amount that might be consumed by the use of OT powder as a dietary supplement. The dietary amount for OT in the HF+OT diet was ~0.17% wt/wt or ~100 mg OT/kg body weight for the mouse. Using the FDA-recommended species conversion of 12.3 (21), this converts to a human dose of 100/12.3 or ~8 mg/kg or 500 mg for a 150-pound person. This amount (500 mg) is very similar to amounts provided by red oak tannin–containing dietary supplements and other typical polyphenol-containing supplements. It may be a limitation of our study that dietary doses equivalent to dietary supplement use, rather than typical consumption of wine, were used. Certainly, to test various dietary amounts would be important in the future. As well, since OT-fed mice consumed less diet, addition of a pair-fed group would allow for further interpretation of the effect of OT consumption.

It may be possible that consuming wines aged in oak barrels likely impart a more robust biological effect than those that see limited or no oak exposure. It has been suggested that a dietary supplement product produced from French oak (*Quercus robur*) extract may help support healthy liver function in people with moderate liver damage due to alcohol consumption (Robuvit; Horphag Research) (22). In 2016, 44 participants aged 45–61 y with moderate functional hepatic failure due to alcohol were randomly selected to consume either 300 mg/d of oak extract or a placebo for 12 wk. They found that the protective effect of the oak extract on liver injury was mediated by its anti-inflammatory and antioxidative activity (23). In a study of red wines aged in oak, the polyphenolic profile of key bioactive substances evolves such that the duration of oak barreling is another variable that may be examined going forward (24).

In closing, we show that the intake of OTs, provided passively in oaked wine or as a dietary supplement, may act in a similar fashion as has been studied for polyphenols consumed in fruits and vegetables. These oak compounds positively impact obesity and metabolic disorders in mice fed an HF diet. Whether these results can be extrapolated to a typical amount of consumption or, importantly, be translated from mice to humans requires further work.

Acknowledgments

The authors' responsibilities were as follows—NS, TG, and TL: designed the research; NS and TL: conducted the research; TL: analyzed data; NS, TL, and TG: wrote the manuscript; NS: had primary responsibility for final content; and all authors: read and approved the final manuscript.

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